JC10 Rec'd PCT/PTO 22 FEB 2002

FORM PTO-1390 (Modified) (REV 11-2000) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER
ASIAP114

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR
10/069825

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/KR99/00488	**26 August 1999**	

TITLE OF INVENTION
MICROORGANISMS AND METHODS FOR PRODUCING THREONINE

APPLICANT(S) FOR DO/EO/US
Hyo Hoon LEE, Jae Chun HAN and Tae Man JUNG

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include itens (5), (6), (9) and (24) indicated below.
4. ☒ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☒ is attached hereto (required only if not communicated by the International Bureau).
   b. ☐ has been communicated by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. ☐ is attached hereto.
   b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
10. ☐ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).
11. ☒ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☒ A copy of the International Search Report (PCT/ISA/210).

Items 13 to 20 below concern document(s) or information included:

13. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☒ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
20. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
21. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
22. ☒ Certificate of Mailing by Express Mail
23. ☐ Other items or information:

PCTUS1/REV03

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR 10/069825	INTERNATIONAL APPLICATION NO. PCT/KR99/00488	ATTORNEY'S DOCKET NUMBER ASIAP114

24. The following fees are submitted:.

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :

		CALCULATIONS	PTO USE ONLY
☒ Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO	**$1040.00**		
☐ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO	**$890.00**		
☐ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO	**$740.00**		
☐ International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4)	**$710.00**		
☐ International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4)	**$100.00**		
ENTER APPROPRIATE BASIC FEE AMOUNT =		**$1,040.00**	
Surcharge of **$130.00** for furnishing the oath or declaration later than months from the earliest claimed priority date (37 CFR 1.492 (e)).	☐ 20 ☒ 30	**$130.00**	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	20 - 20 =	0	x $18.00	**$0.00**	
Independent claims	1 - 3 =	0	x $84.00	**$0.00**	
Multiple Dependent Claims **(check if applicable).**			☐	**$0.00**	
TOTAL OF ABOVE CALCULATIONS =				**$1,170.00**	
☐ Applicant claims small entity status. See 37 CFR 1.27). The fees indicated above are reduced by 1/2.				**$0.00**	
SUBTOTAL =				**$1,170.00**	
Processing fee of **$130.00** for furnishing the English translation later than months from the earliest claimed priority date (37 CFR 1.492 (f)).			☐ 20 ☐ 30 +	**$0.00**	
TOTAL NATIONAL FEE =				**$1,170.00**	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).**			☐	**$0.00**	
TOTAL FEES ENCLOSED =				**$1,170.00**	
				Amount to be: refunded	$
				charged	$

a. ☒ A check in the amount of **$1,170.00** to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. ______________ in the amount of ______________ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 50805/ASIAP114 A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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Peter B. Martine
NAME

32,043
REGISTRATION NUMBER

February 22, 2002
DATE

10/069825
JC13 Rec'd PCT/PTO 22 FEB 2002

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the application of	)	
	)	Examiner: Unassigned
Hyo Hoon LEE et al.	)	
	)	Art Unit: Unassigned
Application No. [To Be Assigned]	)	
	)	Docket No. ASIAP114
Filed: February 22, 2002	)	
	)	February 22, 2002
For: MICROORGANISMS AND METHODS FOR PRODUCING THREONINE	)	

CERTIFICATE OF EXPRESS MAILING

I hereby certify that this paper and the documents and/or fees referred to as attached herein are being deposited with the United States Postal Service on February 22, 2002 in an envelope as "Express Mail Post Office to Addressee" service under 37 CFR § 1.10, Mailing Label Number **EV053657712US**, addressed to the Designated/Elected Office (DO/EO/US), Washington, DC 20231.

Diane Schwanbeck

PRELIMINARY AMENDMENT

Commissioner for Patents
Box PCT
Washington, D.C. 20231

Dear Sir:

This paper is a preliminary amendment regarding International Application No. PCT/KR99/00488, which is now entering the national phase in the U.S. Patent and Trademark Office. Please amend this application as follows:

IN THE CLAIMS:

Please amend claims 2-5 and 8 as indicated below. Attached hereto as separate pages are "marked-up" versions of claims 2-5 and 8 that show the changes made to these claims. For the Examiner's convenience, all pending claims are reproduced below, with those claims that have been amended herein being indicated as such.

1. An L-threonine-producing microorganism having characteristics comprising: requirement of L-methionine for growth; L-isoleucine-leaky for growth; resistance to α-methylserine, diaminosuccinic acid, L-glutamic acid, L-threonine, fermentation mother liquid containing L-threonine, azetidine and dehydroproline; and susceptibility to fluoropyruvate.

2. (Amended) The microorganism of Claim 1, wherein said microorganism has characteristics comprising: growth in minimal medium comprising L-methionine at a concentration from about 50 mg/ℓ to about 400 mg/ℓ but not in minimal medium lacking methionine; more robust growth in minimal medium comprising L-isoleucine at a concentration from about 50 mg/ℓ to about 400 mg/ℓ, than in minimal medium lacking L-isoleucine; growth in medium comprising α-methylserine at a concentration from about 10mM to about 200mM; growth in medium comprising diaminosuccinic acid at a concentration from about 0.5g/L to about 50g/L; growth in medium comprising L-glutamic acid at a concentration from about 50mM to about 500mM; growth in medium comprising L-threonine at a concentration from about 1% to about 13%; growth in medium comprising fermentation mother liquid at a concentration from about 20% to about 80%; growth in medium comprising azetidine at a concentration from about 0.5 g/ℓ to about 5 g/ℓ; growth in medium comprising dehydroproline at a concentration from about 0.5 g/ℓ to about 5 g/ℓ, and inability to grow in medium comprising fluoropyruvate at a concentration from about 10mM to about 200mM.

3. (Amended) The microorganism of Claim 2, wherein said microorganism has characteristics comprising: growth in minimal medium comprising L-methionine at a concentration from about 100 mg/ℓ to about 300 mg/ℓ but not in minimal medium lacking methionine; more robust growth in minimal medium comprising L-isoleucine at a concentration from about 100 mg/ℓ to about 300 mg/ℓ, than in minimal medium lacking L-isoleucine; growth in medium comprising α-methylserine at a concentration from about 20mM to about 100mM; growth in medium comprising diaminosuccinic acid at a concentration from about 1g/L to about 10g/L; growth in medium comprising L-glutamic acid at a concentration from about 100mM to about 300mM; growth in medium comprising L-threonine at a concentration from about 3% to about 10%; growth in medium comprising fermentation mother liquid at a concentration from about 40% to about 70%; growth in medium comprising azetidine at a concentration from about 1 g/ℓ to about 3 g/ℓ; growth in medium comprising dehydroproline at a concentration from about 1 g/ℓ to about 3 g/ℓ, and

inability to grow in medium comprising fluoropyruvate at a concentration from about 20mM to about 100mM.

4. (Amended) The microorganism of Claim 3, wherein said microorganism has characteristics comprising: growth in minimal medium comprising L-methionine at a concentration of about 200 mg/ℓ but not in minimal medium lacking methionine; more robust growth in minimal medium comprising L-isoleucine at a concentration of about 200 mg/ℓ than in minimal medium lacking L-isoleucine; growth in medium comprising α-methylserine at a concentration of about 40mM; growth in medium comprising diaminosuccinic acid at a concentration of about 2.5g/L; growth in medium comprising L-glutamic acid at a concentration of about 240mM; growth in medium comprising L-threonine at a concentration of about 7%; growth in medium comprising fermentation mother liquid at a concentration of about 60%; growth in medium comprising azetidine at a concentration of about 2 g/ℓ; growth in medium comprising dehydroproline at a concentration of about 2 g/ℓ; and inability to grow in medium comprising fluoropyruvate at a concentration of about 40mM.

5. (Amended) The microorganism of Claim 1, wherein said microorganism is a strain of *Escherichia coli.*

6. The microorganism of Claim 5, wherein said microorganism has the Korean Culture Center of Microorganisms deposit number KCCM-10168.

7. The microorganism of Claim 5, wherein said microorganism has all the characteristics of the microorganism having the Korean Culture Center of Microorganisms deposit number KCCM-10168.

8. (Amended) A method of making L-threonine comprising growing a microorganism according to Claim 1 under conditions in which said microorganism produces L-threonine.

Please add the following new claims:

9. (New) The microorganism of Claim 2, wherein said microorganism is a strain of *Escherichia coli.*

10. (New) The microorganism of Claim 3, wherein said microorganism is a strain of *Escherichia coli.*

11. (New) The microorganism of Claim 4, wherein said microorganism is a strain of *Escherichia coli.*

12. (New) A method of making L-threonine comprising growing a microorganism according to Claim 1 under conditions in which said microorganism produces L-threonine.

13. (New) A method of making L-threonine comprising growing a microorganism according to Claim 2 under conditions in which said microorganism produces L-threonine.

14. (New) A method of making L-threonine comprising growing a microorganism according to Claim 3 under conditions in which said microorganism produces L-threonine.

15. (New) A method of making L-threonine comprising growing a microorganism according to Claim 4 under conditions in which said microorganism produces L-threonine.

16. (New) A method of making L-threonine comprising growing a microorganism according to Claim 5 under conditions in which said microorganism produces L-threonine.

17. (New) A method of making L-threonine comprising growing a microorganism according to Claim 6 under conditions in which said microorganism produces L-threonine.

18. (New) A method of making L-threonine comprising growing a microorganism according to Claim 9 under conditions in which said microorganism produces L-threonine.

19. (New) A method of making L-threonine comprising growing a microorganism according to Claim 10 under conditions in which said microorganism produces L-threonine.

20. (New) A method of making L-threonine comprising growing a microorganism according to Claim 11 under conditions in which said microorganism produces L-threonine.

Remarks

Upon entry of this paper, claims 1-20 are pending in this application.

Applicants have amended claims 2-5 and 8, and have added new claims 9-20. These changes have been made to correct typographical errors and to present the claims in a format that does not include improper multiple claim dependencies. As such, these changes do not introduce any new matter.

Applicants respectfully request examination on the merits for claims 1-20. If any additional fees are due in connection with filing this paper, then the Commissioner may charge such fees to Deposit Account No. 50-0805 (Order No. ASIAP114).

Respectfully submitted,
MARTINE & PENILLA, L.L.P.

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MARKED-UP VERSIONS OF AMENDED CLAIMS

2. (Amended) The microorganism of Claim 1, wherein said microorganism has characteristics comprising: growth in minimal medium comprising L-methionine at a concentration from about 50 mg/ℓ to about 400 mg/ℓ but not in minimal medium lacking methionine; more robust growth in minimal medium comprising L-isoleucine at a concentration from about 50 mg/ℓ to about 400 mg/ℓ, than in minimal medium lacking L-isoleucine; growth in medium comprising α-methylserine at a concentration from about 10mM to about 200mM; growth in medium comprising diaminosuccinic acid at a concentration from about 0.5g/L to about 50g/L; growth in medium comprising L-glutamic acid at a concentration from about 50mM to about 500mM; growth in medium comprising L-threonine at a concentration from about 1% to about 13%; growth in medium comprising fermentation mother liquid at a concentration from about 20% to about 80%; growth in medium comprising azetidine at a concentration from about 0.5 g/ℓ to about 5 g/ℓ; growth in medium comprising dehydroproline at a concentration from about 0.5 g/ℓ to about 5 g/ℓ, and inability to grow in medium comprising <u>fluoropyruvate at</u> [fluorpyruvate] a concentration from about 10mM to about 200mM.

3. (Amended) The microorganism of Claim 2, wherein said microorganism has characteristics comprising: growth in minimal medium comprising L-methionine at a concentration from about 100 mg/ℓ to about 300 mg/ℓ but not in minimal medium lacking methionine; more robust growth in minimal medium comprising L-isoleucine at a concentration from about 100 mg/ℓ to about 300 mg/ℓ, than in minimal medium lacking L-isoleucine; growth in medium comprising α-methylserine at a concentration from about 20mM to about 100mM; growth in medium comprising diaminosuccinic acid at a concentration from about 1g/L to about 10g/L; growth in medium comprising L-glutamic acid at a concentration from about 100mM to about 300mM; growth in medium comprising L-threonine at a concentration from about 3% to about 10%; growth in medium comprising fermentation mother liquid at a concentration from about 40% to about 70%; growth in medium comprising azetidine at a concentration from about 1 g/ℓ to about 3 g/ℓ; growth in medium comprising dehydroproline at a concentration from about 1 g/ℓ to about 3 g/ℓ, and inability to grow in medium comprising <u>fluoropyruvate at</u> [fluorpyruvate] a concentration from about 20mM to about 100mM.

4. (Amended) The microorganism of Claim 3, wherein said microorganism has characteristics comprising: growth in minimal medium comprising L-methionine at a concentration of about 200 mg/ℓ but not in minimal medium lacking methionine; more robust growth in minimal medium comprising L-isoleucine at a concentration of about 200 mg/ℓ than in minimal medium lacking L-isoleucine; growth in medium comprising α-methylserine at a concentration of about 40mM; growth in medium comprising diaminosuccinic acid at a concentration of about 2.5g/L; growth in medium comprising L-glutamic acid at a concentration of about 240mM; growth in medium comprising L-threonine at a concentration <u>of</u> [from] about 7%; growth in medium comprising fermentation mother liquid at a concentration of about 60%; growth in medium comprising azetidine at a concentration of about 2 g/ℓ; growth in medium comprising dehydroproline at a concentration of about 2 g/ℓ; and inability to grow in medium comprising <u>fluoropyruvate at</u> [fluorpyruvate] a concentration of about 40mM.

5. (Amended) The microorganism of <u>Claim 1</u> [any one of Claims 1-4], wherein said microorganism is a strain of *Escherichia coli.*

8. (Amended) A method of making L-threonine comprising growing a microorganism according to <u>Claim 1</u> [any one of the foregoing claims] under conditions in which said microorganism produces L-threonine.

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MICROORGANISMS AND METHODS FOR PRODUCING THREONINE

Background of the Invention

Field of the Invention

The present invention relates to microorganisms and methods for producing L-threonine. In particular, the present invention relates to the production of L-threonine using microorganisms, and *Escherichia coli* strains in particular, which require L-methionine, are L-isoleucine-leaky for growth, are resistant to α-methylserine, diaminosuccinic acid, L-glutamic acid, L-threonine, medium containing 60% of L-threonine fermentation mother liquid (which contains more than 9.0% threonine), azetidine and dehydroproline, and which are susceptible to fluoropyruvate.

Description of the Related Art

L-threonine, an essential amino acid, is a second limited amino acid of rice. As is well known, L-threonine is used as a component for, e.g., amino acid transfusion liquid or general amino acid tablets, and as a nutrient. Recently, there has been a great increase in the demand for L-threonine because it, together with L-lysine, is used as an additive in feedstuff.

Japanese Pat. Publication No. Heisei 5-10076 teaches use of recombinant DNA which contains the genetic information for asparto kinase, homoserine kinase, homoserine dehydrogenase, and threonine synthase in production of threonine from a L-threonine-producing *Serratia* sp. Japanese Pat. Publication No. Heisei 1-289493 discloses that a DNA taken from a *Providencia* sp. resistant to methionine metabolic antagonist is genetically engineered and used to increase the productivity of L-threonine. In order to produce L-threonine, a threonine metabolic antagonist-resistant *Escherichia* sp. which requires methionine or diaminopimelic acid for growth has been used (Japanese Pat. Publication No. Sho. 56-10037). A strain which can grow in medium containing L-serine and ethionine has also been used (EP 91103569.9).

Summary of the Invention

The present invention relates to novel microorganisms and methods of producing large quantities of L-threonine using the microorganisms. The present microorganisms are derived from the microorganism deposited in the Korean Culture Center of Microorganisms, College of Engineering, Yonsei University, Sodaemun gu, Seoul 120-749,

Republic of Korea, on July 16, 1998, having the deposition number KCCM-10132. KCCM-10132 has also been described in PCT Application Number PCT/KR 98/00340.

KCCM-10132, the parent strain of the present microorganisms, requires both L-methionine and L-isoleucine, is resistant to α-methylserine, diaminosuccinic acid, L-glutamic acid and L-threonine, and is susceptible to fluoropyruvate. KCCM 10132 also requires diaminopimelic acid.

To obtain the present microorganisms, KCCM-10132 was mutated and cells which were resistant to L-threonine, able to grow on medium containing 60% of L-threonine fermentation mother liquid (which contains more than 9.0% threonine), azetidine and dehydroproline were selected. As used herein, the terminology "able to grow on medium containing L-threonine fermentation mother liquid" means that the microorganism is able to grow on minimal agar plates which contain the production medium for the parent strain KCCM10132, the ingredients of which are listed in Example 2. The production medium for the parent strain KCCM 10132 contains glucose at a concentration of 10%, corn steep liquor at a concentration of 3%, potassium dihydrogen phosphate at a concentration of 0.1%, ferrous sulfate at a concentration of 2 mg/ℓ, manganese sulfate at a concentration of 2 mg/ℓ, ammonium sulfate at a concentration of 0.05%, urea at a concentration of 0.6%, L-methionine at a concentration of 200 mg/ℓ and pH 7.0 and L-isoleucine at a concentration of 200 mg/ℓ. To prepare the "L-threonine fermenation mother liquid" the parent strain KCCM10132 is grown in the preceding medium for 100 hours at 30 degrees Celsius. When cultured in media containing a high concentration of glucose, the present microorganisms accumulate large quantities of L-threonine in the culture.

Detailed Description of the Preferred Embodiments

The novel strain of the present invention, which has been deposited in the Korean Culture Center of Microorganisms and has been assigned deposit number KCCM-10168, can grow well in the presence of fermentation mother liquid in which the strain was grown, L-azetidine-2-carboxylic acid and 3,4-dehydro-DL-proline. In other words, KCCM-10168 is resistant to fermentation mother liquid in which the strain was grown, L-azetidine-2-carboxylic acid (hereinafter "azetidine") and 3,4-dehydro-DL-proline(hereinafter "dehydroproline").

As discussed above, KCCM-10168 was derived from a strain deposited at the

Korean Culture Center of Microorganisms under deposit number KCCM-10132. The strains of the present invention may be obtained by treating KCCM-10132 with UV or with chemical mutagens, such as NTG(N-methyl-N'-nitro-N-nitroso guanidine) and DES(diethylsulfate). Following mutagenesis, the cells were streaked on minimal agar plates containing 60% of L-threonine fermentation mother liquid(more than 9.0% of threonine) to select L-threonine fermentation mother liquid-resistant colonies.

The selected colonies were streaked on complete agar plates containing 60% of L-threonine fermentation mother liquid, 2g/ℓ azetidine and 2g/ℓ dehydroproline and cultured at 37 ℃ for 2~3days. The complete agar plates included yeast extract 1.0%, peptone 1.0%, beef broth 0.3%, NaCl 0.5% and glucose 0.5% at pH 7.0.

Thereafter, replicas of the colonies grown were made on a minimal agar plate containing 60% of L-threonine fermentation mother liquid, 2g/ℓ azetidine and 2g/ℓ dehydroproline and a minimal agar plate devoid of these components. Of the colonies which survived on the agar plate containing 60% of L-threonine fermentation mother liquid, 2g/ℓ azetidine and 2g/ℓ dehydroproline, those which were clearly grown were separated, and their characteristics were compared with those of the parent strain KCCM-10132. The minimal plates on which the microbiological properties of the mutant and parent strains were compared included 1.0% glucose, 0.2% ammonium sulfate, 0.1% potassium dihydrogen phosphate, 0.02% magnesium sulfate at pH 7.3, and 2% agar. Diaminopimelic acid at 100 ㎎/ℓ, L-methionine at 200 ㎎/ℓ and L-isoleucine at 200 ㎎/ℓ were respectively used in order to determine whether they were needed for the growth of the novel strain.

DSM9906 is a strain obtained using the above procedure. DSM9906 was deposited in the Korea Culture Center of Microorganisms, College of Engineering, Yonsei University, Sodaemun gu, Seoul 120-749, Republic of Korea, on July 29, 1999 and was assigned Deposition No. KCCM-10168. While the following discussion utilizes KCCM-10168 as an example of the microorganisms of the present invention, it will be appreciated that other strains, and in particular other *E coli* strains, which have the properties of KCCM-10168 may be used to produce L-threonine. In particular, the *E. coli* strains may be derived from KCCM-10132. Alternatively, the microorganisms may be from species other than *E. coli*, including strains of *Brevibacteria* or *Corynebacteria*.

For example, to select a microorganism which requires L-methionine for growth,

the microorganism is mutagenized as described above. After mutagenesis, the cells are streaked on complete agar plates comprising 1% yeast extract, 1% peptone, 0.3% beef broth, 0.5% NaCl, 0.5% glucose, and 2% agar at pH 7.0. The colonies are replica plated on minimal plates containing 5.0% fructose, 1.4% ammonium sulfate, 0.2% potassium dihydrogen phosphate, 0.1% magnesium sulfate, a sufficient amount of any additional compounds required by the microorganism to permit growth, and 2% agar at pH 7.3 with or without L-methionine in order to identify colonies which grow in the presence of L-methionine but not in the absence of L-methionine. The L-methionine may be present at any concentration which is sufficient to differentiate strains which require L-methionine from strains which do not require L-methionine. For example, the L-methionine may be present at from about 50 mg/ℓ to about 400 mg/ℓ, preferably from about 100 mg/ℓ to about 300 mg/ℓ, and more preferably at about 200 mg/ℓ. Strains which grow in the presence of L-methionine but not in its absence may be used in conjunction with the present invention.

For example, to select a microorganism which is leaky for L-isoleucine, the microorganism is mutagenized as described above. After mutagenesis, the cells are streaked on complete agar plates comprising 1% yeast extract, 1% peptone, 0.3% beef broth, 0.5% NaCl, 0.5% glucose, and 2% agar at pH 7.0. The colonies are replica plated on minimal plates containing 5.0% fructose, 1.4% ammonium sulfate, 0.2% potassium dihydrogen phosphate, 0.1% magnesium sulfate, a sufficient amount of any additional compounds required by the microorganism to permit growth and 2% agar at pH 7.3 with or without L-isoleucine in order to identify colonies which grow slowly in the absence of L-isoleucine but which exhibit more robust growth in the presence of L-isoleucine. The L-isoleucine may be present at any concentration which is sufficient to identify strains which grow slowly in the absence of L-isoleucine from strains but which exhibit more robust growth in its presence. For example, growth on plates containing L-isoleucine at from about 50 mg/ℓ to about 400 mg/ℓ, preferably from about 100 mg/ℓ to about 300 mg/ℓ, and more preferably at about 200 mg/ℓ can be compared to growth on plates lacking L-isoleucine. Strains which grow slowly in the absence of L-isoleucine but more robustly in its presence may be used in conjunction with the present invention.

To select a microorganism which is resistant to α-methylserine, the microorganism is mutagenized as described above. After mutagenesis, the cells are streaked on complete

agar plates comprising 1% yeast extract, 1% peptone, 0.3% beef broth, 0.5% NaCl, 0.5% glucose, α-methylserine and 2% agar at pH7.0. The α-methylserine may be present at any concentration which is sufficient to differentiate strains which are resistant to α-methylserine from strains which are sensitive to α-methylserine. For example, growth on plates containing α-methylserine at from about 10mM to about 200mM, preferably from about 20mM to about 100mM, and more preferably at about 40mM indicates that the strain is resistant to α-methylserine. The resulting colonies are then replica plated on minimal plates containing 5.0% fructose, 1.4% ammonium sulfate, 0.2% potassium dihydrogen phosphate, 0.1% magnesium sulfate, a sufficient amount of any additional compounds required by the microorganism to permit growth, and 2% agar at pH 7.3 with or without α-methylserine at the concentration used in the complete agar plates in order to identify colonies which are resistant to α-methylserine. Strains which grow in the presence of α-methylserine may be used in conjunction with the present invention.

To select a microorganism which is resistant to diaminosuccinic acid, the microorganism is mutagenized as described above. After mutagenesis, the cells are streaked on complete agar plates comprising 1% yeast extract, 1% peptone, 0.3% beef broth, 0.5% NaCl, 0.5% glucose, diaminosuccinic acid and 2% agar at pH7.0. The diaminosuccinic acid may be present at any concentration which is sufficient to differentiate strains which are resistant to diaminosuccinic acid from strains which are sensitive to diaminosuccinic acid. For example, growth on plates containing diaminosuccinic acid at from about 0.5g/L to about 50g/L, preferably from about 1 g/L to about 10g/L, and more preferably at about 2.5g/L indicates that the strain is resistant to diaminosuccinic acid. The colonies are replica plated on minimal plates containing 5.0% fructose, 1.4% ammonium sulfate, 0.2% potassium dihydrogen phosphate, 0.1% magnesium sulfate, a sufficient amount of any additional compounds required by the microorganism to permit growth, and 2% agar at pH 7.3 with or without diaminosuccinic acid at the concentration used in the complete agar plates in order to identify colonies which are resistant to diaminosuccinic acid. Strains which grow in the presence of diaminosuccinic acid may be used in conjunction with the present invention.

To select a microorganism which is resistant to L-glutamic acid, the microorganism is mutagenized as described above. After mutagenesis, the cells are streaked on complete agar plates comprising 1% yeast extract, 1% peptone, 0.3% beef broth, 0.5% NaCl, 0.5%

glucose, L-glutamic acid and 2% agar at pH7.0. The L-glutamic acid may be present at any concentration which is sufficient to differentiate strains which are resistant to L-glutamic acid from strains which are sensitive to L-glutamic acid. For example, growth on plates containing L-glutamic acid at from about 50mM to about 500mM, preferably from about 100mM to about 300mM, and more preferably at about 240mM indicates that the strain is resistant to L-glutamic acid. The colonies are replica plated on minimal plates containing 5.0% fructose, 1.4% ammonium sulfate, 0.2% potassium dihydrogen phosphate, 0.1% magnesium sulfate, a sufficient amount of any additional compounds required by the microorganism to permit growth, and 2% agar at pH 7.3 with or without L-glutamic acid at the concentration used in the complete agar plates in order to identify colonies which are resistant to L-glutamic acid. Strains which grow in the presence of L-glutamic acid may be used in conjunction with the present invention.

To select a microorganism which is resistant to L-threonine, the microorganism is mutagenized as described above. After mutagenesis, the cells are streaked on complete agar plates comprising 1% yeast extract, 1% peptone, 0.3% beef broth, 0.5% NaCl, 0.5% glucose. L-threonine and 2% agar at pH7.0. The L-threonine may be present at any concentration which is sufficient to differentiate strains which are resistant to L-threonine from strains which are sensitive to L-threonine. For example, growth on plates containing L-threonine at from about 1% to about 13%, preferably from about 3% to about 10%, and more preferably at about 7% indicates that the strain is resistant to L threonine. The colonies are replica plated on minimal plates containing 5.0% fructose, 1.4% ammonium sulfate, 0.2% potassium dihydrogen phosphate, 0.1% magnesium sulfate, a sufficient amount of any additional compounds required by the microorganism to permit growth, and 2% agar at pH 7.3 with or without L-threonine at the concentration used in the complete agar plates in order to identify colonies which are resistant to L-threonine. Strains which grow in the presence of L-threonine may be used in conjunction with the present invention.

To select a microorganism which is resistant to L-threonine fermentation mother liquid, the microorganism is mutagenized as described above. After mutagenesis, the cells are streaked on complete agar plates comprising 1% yeast extract, 1% peptone, 0.3% beef broth, 0.5% NaCl, 0.5% glucose, L-threonine fermentation mother liquid and 2% agar at pH7 0 The L-threonine fermentation mother liquid may be present at any concentration which is sufficient to differentiate strains which are resistant to L-threonine fermentation mother liquid from strains which are sensitive to L-threonine fermentation mother liquid.

For example, the plates may contain from about 20 to about 80%, more preferably from aobut 40 to about 70%, and in particular 60% L-threonine fermentation mother liquid. The colonies are replica plated on minimal plates containing 5.0% fructose, 1.4% ammonium sulfate, 0.2% potassium dihydrogen phosphate, 0.1% magnesium sulfate, a sufficient amount of any additional compounds required by the microorganism to permit growth, and 2% agar at pH 7.3 with or without L-threonine fermentation mother liquid at the concentration used in the complete agar plates in order to identify colonies which are resistant to L-threonine fermentation mother liquid. Strains which grow in the presence of L-threonine fermentation mother liquid may be used in conjunction with the present invention.

To select a microorganism which is resistant to azetidine, the microorganism is mutagenized as described above. After mutagenesis, the cells are streaked on complete agar plates comprising 1% yeast extract, 1% peptone, 0.3% beef broth, 0.5% NaCl, 0.5% glucose, azetidine and 2% agar at pH7.0. The azetidine may be present at any concentration which is sufficient to differentiate strains which are resistant to azetidine from strains which are sensitive to azetidine For example, growth on plates containing azetidine at from about 0.5 g/ ℓ to about 5 g/ ℓ, preferably from about 1 g/ ℓ to about 3 g/ ℓ, and more preferably at about 2 g/ ℓ indicates that the strain is resistant to azetidine. The colonies are replica plated on minimal plates containing 5.0% fructose, 1.4% ammonium sulfate, 0.2% potassium dihydrogen phosphate, 0.1% magnesium sulfate, a sufficient amount of any additional compounds required by the microorganism to permit growth, and 2% agar at pH 7.3 with or without azetidine at the concentration used in the complete agar plates in order to identify colonies which are resistant to azetidine. Strains which grow in the presence of azetidine may be used in conjunction with the present invention.

To select a microorganism which is resistant to dehydroproline, the microorganism is mutagenized as described above. After mutagenesis, the cells are streaked on complete agar plates comprising 1% yeast extract, 1% peptone, 0.3% beef broth, 0.5% NaCl, 0.5% glucose, dehydroproline and 2% agar at pH7.0. The dehydroproline may be present at any concentration which is sufficient to differentiate strains which are resistant to dehydroproline from strains which are sensitive to dehydroproline. For example, growth on plates containing dehydroproline at from about 0.5 g/ ℓ to about 5 g/ ℓ, preferably

from about 1 g/ ℓ to about 3 g/ ℓ, and more preferably at about 2 g/ ℓ indicates that the strain is resistant to dehydroproline. The colonies are replica plated on minimal plates containing 5.0% fructose, 1.4% ammonium sulfate 0.2% potassium dihydrogen phosphate, 0.1% magnesium sulfate, a sufficient amount of any additional compounds required by the microorganism to permit growth, and 2% agar at pH 7.3 with or without dehydroproline at the concentration used in the complete agar plates in order to identify colonies which are resistant to azetidine. Strains which grow in the presence of dehydroproline may be used in conjunction with the present invention.

To select a microorganism which is sensitive to fluoropyruvate, the microorganism is mutagenized as described above. After mutagenesis, the cells are streaked on complete agar plates comprising 1% yeast extract, 1% peptone, 0.3% beef broth, 0.5% NaCl, 0.5% glucose, and 2% agar at pH7.0. The colonies are replica plated on minimal plates containing 5.0% fructose, 1.4% ammonium sulfate, 0.2% potassium dihydrogen phosphate, 0.1% magnesium sulfate, a sufficient amount of any additional compounds required by the microorganism to permit growth, and 2% agar at pH 7.3 with or without fluoropyruvate. The fluoropyruvate may be present at any concentiation which is sufficient to differentiate strains which are sensitive to fluoropyruvate from strains which are resistant to fluoropyruvate. For example. lack of growth on plates containing fluoropyruvate at from about 10mM to about 200mM, preferably from about 20mM to about 100mM, and more preferably at about 40mM indicates that the strain is sensitive to fluoropyruvate. Strains which grow in absence of fluoropyruvate but not in the presence of fluoropyruvate may be used in conjunction with the present invention.

It will be appreciated that each of the above selection procedures need not be performed in individual steps. Instead, after mutagenesis, the microorganisms may be plated on complete agar which selects for more than one characteristic simultaneously. For example, two or more characteristics may be simultaneosly selected. For example, to select cells which are resistant to α-methylserine, diaminosuccinic acid, L-glutamic acid, L-threonine, L-threonine fermentation mother liquid, azetidine, and dehydroproline, these each of these compositions may be included in the complete agar plates on which the mutagenized cells are streaked at the concentrations provided above. Thereafter, the mutagenized cells may be plated on minimal agar plates containing each of these compositions at the concentrations provided above.

It will also be appreciated that mutagenesis need not be separately performed for each characteristic to be selected. Rather, after mutagenesis, microorganisms which have a desired characteristic may be identified as provided above. Thereafter, the identified microorganisms may be selected for additional desired characteristics as described above.

As shown in Table 1, the novel strain KCCM-10168 is resistant to 60% of L-threonine fermentation mother liquid, 2g/ ℓ azetidine and 2g/ ℓ dehydroproline. As shown in Table 2, KCCM-10168 retains most of the characteristics of the parent strain, including a requirement for L-methione and resistance to L-threonine and L-glutamic acid. However, unlike the parent strain, KCCM-10168 is isoleucine-leaky and does not require diaminopimelic acid.

Table 1. Growth of the novel strain KCCM-10168 in the broths containing 60% of L-threonine fermentation mother liquid, azetidine and dehydroproline.

Concentration			Strains	
L-threonine fermentation mother liquid(%)	Azetidine(g/ ℓ)	Dehydroproline (g/ ℓ)	Parent strain (KCCM-10132)	novel strain DSM9906 (KCCM-10168)
0	0	0	1.804	1.821
60	1	1	0.053	1.675
	2	2	0.048	1.587
	4	4	0.049	0.501
80	1	1	0.058	0.092
	2	2	0.064	0.089
	4	4	0.059	0.077

Note: Growth of the strains was measured by absorbance at 610 nm after culturing them for 24 hours in minimal broth containing above three compounds.

Table 2. The comparison of the characteristics of DSM9906(KCCM-10168) and Parent strain (KCCM-10132).

Concentration						Strains			
L-Met (mg/ℓ)	L-Ile (mg/ℓ)	DAPA (mg/ℓ)	Fer. Liquid 60%	Azeti.	De Hydro proline	Parent strain (KCCM-10132)		DSM9906 (KCCM-10168)	
						Min. broth	Com -plete broth	Min. broths	Com -plete broth
200	-	-	-	-	-	-	+++	+	+++
-	200	-	-	-	-	-	+++	-	+++
200	200	-	-	-	-	+	+++	+++	+++
200	200	100	-	-	-	+++	+++	+++	+++
200	200	100	add	2g/ℓ	2g/ℓ	-	+	+++	+++

Note : Growth state after being cultured in broth containing above six compounds. (-:no growth, +:growth, ++:good growth, +++:very good growth)

The growth and yield of L-threonine obtained with DSM9906 (KCCM-10168) was compared to those obtained with the parent strain KCCM-10132 at different glucose concentrations and the results are shown in Table 3. As shown in Table 3, KCCM-10168 provided a greater yield of L-threonine than KCCM-10132 in a high concentration of glucose.

Table 3. Comparison of the growth and productivity between DSM9906 (KCCM-10168) and KCCM-10132.

L-Glu Conc.		Strains	
		KCCM-10132	DSM9906 (KCCM-10168)
5.0%	Growth[1]	0.555	0.567
	L-threonine[2]	12.53	12.61
7.0%	Growth	0.608	0.613
	L-threonine	16.92	17.34
10.0%	Growth	0.852	0.866
	L-threonine	19.86	22.97
12.5%	Growth	0.590	0.861
	L-threonine	13 09	22.23

Note[1] : 50-fold diluted solutions of the cultures incubated for 36~72 hours in production media(Example 1) were measured by absorbance at 610nm(Beckman DU-70)
Note[2] : L-threonine accumulated in cultures was measured using an automatic amino acid analyzer(Hitachi L-8500A)

Example 1 Production of L-threonine using DSM9906 (KCCM-10168)

• Strain used : DSM9906 (KCCM-10168)

• Pre-culture medium composition : Glucose 0.5%, Yeast Extract 1.0%, Peptone 1.0%, NaCl 0.5%, Beef broth 0.3%, pH 7.0

• Production medium composition : Glucose 12.5%, Corn steep liquor 3%, Potassium dihydrogen phosphate 0.1%, Ferrous sulfate 2 mg/ ℓ , Manganese sulfate 2 mg/ ℓ , Ammonium sulfate 0.5%, L-Methionine 200 mg/ ℓ and Calcium carbonate 5%(separately sterilized), pH 7.0. In the case of the parent strain KCCM-10132, L-Isoleucine was added at 200 mg/ ℓ .

• Pre-Culturing : 5 mℓ of the pre-culture medium was aliquoted to 18Φ × 185mm test tubes and autoclaved at 121 ℃ for 15min. under pressure. After being cooled, the aliquots were inoculated with the novel strain DSM9906 (KCCM-10168) by use of a sterilized metal loop. They were incubated at 30℃ for 20 hours with shaking at 120 cycles per min.

• Production Culturing : 70 mℓ aliquots of the threonine production media were placed in 500 mℓ Sakaguchi flasks and autoclaved at 121℃ for 15 min. under pressure. After being cooled, the aliquots of the autoclaved threonine production media were inoculated with the pre-cultures of DSM9906(KCCM-10168) at a level of 1%. The strain was incubated at 30℃ for 72 hours with shaking at 120 cycles per min. After fermentation, L-threonine was found to be accumulated at an amount of 22.23 mg/mℓ in the novel strain DSM9906(KCCM-10168) culture. When the above procedure was performed using the parent strain KCCM-10132, L-threonine was found to be accumulated at an amount of 13.09 mg/mℓ.

Example 2

• Strain used : DSM9906 (KCCM-10168)

• Primary pre-culture medium composition : Same as the Pre-culture medium composition of Example 1.

• Secondary Pre-culture medium composition : Glucose 2%, Corn steep liquor 3%, Potassium dihydrogen phosphate 0.1%, Ferrous sulfate 2 mg/ ℓ , Manganese sulfate 2 mg/ ℓ , Ammonium sulfate 0.05%, Urea 0.6%, L-Methionine 200 mg/ ℓ , pH 7.0

• Production medium composition : Glucose 10%, Corn steep liquor 3%, Potassium dihydrogen phosphate 0.1%, Ferrous sulfate 2 mg/ ℓ , Manganese sulfate 2 mg/ ℓ , Ammonium sulfate 0.05%, Urea 0.6%, L-Methionine 200 mg/ ℓ and pH 7.0. In the case of

the parent strain KCCM-10132, L-Isoleucine was added at 200 ㎎/ ℓ .

• Pre-Culturing : A primary pre-culture of DSM9906(KCCM-10168) was obtained in the same manner as that of Example 1. It was inoculated at 1% in 50 ㎖ aliquots of the secondary pre-culture media in Sakaguchi flasks, which had been autoclaved at 121 ℃ for 15min. Incubation was carried out at 30 ℃ for 24 hours with shaking at 120 cycles per min, to give a secondary pre-culture.

• Production Culturing : 2 ℓ of the production media were bottled in a 5 ℓ fermentation bath and then autoclaved at 121 ℃ for 15 min. under pressure. The secondary culture of DSM9906 (KCCM-10168) was inoculated at 5~10% and incubated at 30 ℃ for 100 hours with aeration at 0.8~1.5vvm and stirring at 550 rpm. Glucose were added so as to maintain the glucose concentration of the media at 1~3%. The media were adjusted into pH 6.5~7.0 with ammonia water. After fermentation, L-threonine was found to be accumulated at an amount of 110.20 ㎎/㎖ in the DSM9906 (KCCM-10168) culture. In the same manner as the above, L-threonine was produced from the parent strain KCCM-10132 and measured to be 95.24 ㎎/㎖. 1 ℓ of each of the cultures was centrifuged to harvest the bacteria. The supernatant was passed through an ion exchange resin to adsorb L-threonine, eluted and purified to yield L-threonine crystals at an amount of 104.7 ㎎/㎖ from the culture of DSM9906 and 90.5 ㎎/㎖ from the culture of KCCM-10132. L-Isoleucine 200 ㎎/㎖ was added in the secondary pre-culture medium and production culturing of the parent strain KCCM-10132.

[illegible]

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

To. DAESANG
52 1, KAYANG-DONG,
KANGSEO-KU,
Seoul, 157-200
Korea

RECEIPT IN THE CASE OF AN ORIGINAL
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR : Escherichia coli DSM9906	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: KCCM 10168
II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION	
The microorganism identified under I above was accompanied by : [ ] a scientific description [ ] a proposed taxonomic designation (Mark with a cross where applicable)	
III. RECEIPT AND ACCEPTANCE	
This International Depositary Authority accepts the microorganism identified under I above, which was received by it on July. 22. 1999 (date of the original deposit) [1]	
IV. INTERNATIONAL DEPOSITARY AUTHORITY	
Name : Korean Culture Center of Microorganisms Address : 361-221, Yurim B/D Hongje-1-dong, Seodaemun-gu SEOUL 120-091 Republic of Korea	Signature(s) of person(s) having the power to represent the International Depositary Authority of of authorized official(s) : Date: July. 29. 1999

1 Where Rule 6.4(d) applies, such date is the date on which the status of international depositary authority was acquired : where a deposit made outside the Budapest Treaty after the acquisition of the status of international depositary authority is converted into a deposit under the Budapest Treaty, such date is the date on which the microorganism was received by the international depositary authority.

Form BP/4

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WHAT IS CLAIMED IS:

1. An L-threonine-producing microorganism having characteristics comprising: requirement of L-methionine for growth; L-isoleucine-leaky for growth; resistance to α-methylserine, diaminosuccinic acid, L-glutamic acid, L-threonine, fermentation mother liquid containing L-threonine, azetidine and dehydroproline; and susceptibility to fluoropyruvate.

2. The microorganism of Claim 1, wherein said microorganism has characteristics comprising: growth in minimal medium comprising L-methionine at a concentration from about 50 mg/ℓ to about 400 mg/ℓ but not in minimal medium lacking methionine; more robust growth in minimal medium comprising L-isoleucine at a concentration from about 50 mg/ℓ to about 400 mg/ℓ, than in minimal medium lacking L-isoleucine; growth in medium comprising α-methylserine at a concentration from about 10mM to about 200mM; growth in medium comprising diaminosuccinic acid at a concentration from about 0.5g/L to about 50g/L; growth in medium comprising L-glutamic acid at a concentration from about 50mM to about 500mM; growth in medium comprising L-threonine at a concentration from about 1% to about 13%; growth in medium comprising fermentation mother liquid at a concentration from about 20% to about 80%; growth in medium comprising azetidine at a concentration from about 0.5 g/ℓ to about 5 g/ℓ; growth in medium comprising dehydroproline at a concentration from about 0.5 g/ℓ to about 5 g/ℓ, and inability to grow in medium comprising fluorpyruvate a concentration from about 10mM to about 200mM.

3. The microorganism of Claim 2, wherein said microorganism has characteristics comprising: growth in minimal medium comprising L-methionine at a concentration from about 100 mg/ℓ to about 300 mg/ℓ but not in minimal medium lacking methionine; more robust growth in minimal medium comprising L-isoleucine at a concentration from about 100 mg/ℓ to about 300 mg/ℓ, than in minimal medium lacking L-isoleucine; growth in medium comprising α-methylserine at a concentration from about 20mM to about 100mM; growth in medium comprising diaminosuccinic acid at a concentration from about 1g/L to about 10g/L; growth in medium comprising L-glutamic acid at a concentration from about 100mM to about 300mM; growth in medium comprising L-threonine at a concentration from about 3% to about 10%; growth in

medium comprising fermentation mother liquid at a concentration from about 40% to about 70%; growth in medium comprising azetidine at a concentration from about 1 g/ ℓ to about 3 g/ ℓ ; growth in medium comprising dehydroproline at a concentration from about 1 g/ ℓ to about 3 g/ ℓ , and inability to grow in medium comprising fluorpyruvate a concentration from about 20mM to about 100mM.

4. The microorganism of Claim 3, wherein said microorganism has characteristics comprising: growth in minimal medium comprising L-methionine at a concentration of about 200 mg/ ℓ but not in minimal medium lacking methionine; more robust growth in minimal medium comprising L-isoleucine at a concentration of about 200 mg/ ℓ than in minimal medium lacking L-isoleucine; growth in medium comprising α-methylserine at a concentration of about 40mM; growth in medium comprising diaminosuccinic acid at a concentration of about 2.5g/L; growth in medium comprising L-glutamic acid at a concentration of about 240mM; growth in medium comprising L-threonine at a concentration from about 7%; growth in medium comprising fermentation mother liquid at a concentration of about 60%; growth in medium comprising azetidine at a concentration of about 2 g/ ℓ ; growth in medium comprising dehydroproline at a concentration of about 2 g/ ℓ , and inability to grow in medium comprising fluorpyruvate a concentration of about 40mM.

5. The microorganism of any one of Claims 1-4, wherein said microorganism is a strain of *Escherichia coli*.

6. The microorganism of Claim 5, wherein said microorganism has the Korean Culture Center of Microorganisms deposit number KCCM-10168.

7. The microorganism of Claim 5, wherein said microorganism has all the characteristics of the microorganism having the Korean Culture Center of Microorganisms deposit number KCCM-10168.

8. A method of making L-threonine comprising growing a microorganism according to any one of the foregoing claims under conditions in which said microorganism produces L-threonine.

Abstract of the Disclosure

The present invention relates to methods and microorganisms for producing L-threonine. In particular, the present invention relates to the production of L-threonine using microorganisms, and *Escherichia coli* strains in particular, which require L-methionine for growth and are L-isoleucine-leaky, are resistant to α-methylserine, diaminosuccinic acid, L-glutamic acid, L-threonine, medium containing 60% of L-threonine fermentation mother liquid, azetidine and dehydroproline, and which are susceptible to fluoropyruvate.

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DECLARATION AND POWER OF ATTORNEY FOR ORIGINAL U.S. PATENT APPLICATION

Attorney Docket No.	ASIAP114
First Named Inventor:	LEE, Hyo Hoon
U.S. Application No.	10/069,825
Filing Date:	February 22, 2002

As a below-named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe that I am an original, first and joint inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled: MICROORGANISMS AND METHODS FOR PRODUCING THREONINE, the specification of which,

(check one) 1. ☐ is attached hereto.

2. ☐ was filed on ______________________ as U.S. Application Serial No. ______________ and was amended on ______________.

3. ☒ was filed on August 26, 1999 as International PCT Application Serial No. PCT/KR99/00488 and was amended on February 22, 2002.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, CFR § 1.56, including for continuation-in-part applications, material information which became available between the filing date of the prior application and the national or PCT international filing date of the continuation-in-part application.

I hereby claim foreign priority benefits under Title 35, United States code, § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's or plant breeder's rights certificate(s), or § 365(a) of any PCT International application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's or plant breeder's rights certificate(s), or PCT International application having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s)

(Appl. No.)	(Country)	(Filing Date)	Priority Benefits Claimed?
			☐Yes ☐No
			☐Yes ☐No

9 And I hereby appoint the law firm of Martine & Penilla, L.L.P., including **Peter B. Martine (Reg. No. 32,043), Albert S. Penilla (Reg. No. 39,487), Joe A. Brock II (Reg. No. 46,021), Chester E. Martine, Jr. (Reg. No. 19,711), Edmund H. Mizumoto (Reg. No. 46,938), Rick von Wohld (Reg. No. 48,018), Michael L. Gencarella (Reg. No. 44,703), George Brian Leavell (Reg. No. 45,436), and Lygeri Kokkinakos (Reg. No. 42,756)** as my principal attorneys to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

1-00

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